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| 1 | 2 | ("5854023").PN. | USPAT; US-PGPUB; EPO; JPO; DERWENT | 2003/04/29 15:04 |
| 7 | 3 | S-adenosyl-5-homocysteine ADJ hydrolase | USPAT; US-PGPUB; EPO; JPO; DERWENT | 2003/04/29 15:06 |
| 13 | 93 | homocysteine ADJ hydrolase | USPAT; US-PGPUB; EPO; JPO; DERWENT | 2003/04/29 15:06 |
| 23 | 18 | HART NEAR Derek | USPAT; US-PGPUB; EPO; JPO; DERWENT | 2003/04/29 16:10 |
| 29 | 14 | (US-6376210-\$ or US-4148888-\$ or US-6432666-\$ or US-6479247-\$ or US-5876917-\$).did. or (US-20020035078-\$ or US-20020155510-\$).did. or (WO-9814562-\$).did. or (JP-60180599-\$ or JP-60030679-\$).did. or (US-5854023-\$ or US-20020035078-\$ or US-4148888-\$ or US-6037524-\$).did. | USPAT; US-PGPUB; EPO; JPO; DERWENT | 2003/04/29 16:11 |



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(54) **ENZYME HAVING
S-ADENOSYL-L-HOMOCYSTEINE
HYDROLASE (AHCY) TYPE ACTIVITY**

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(57) ABSTRACT

Enzyme having AHCY-type activity and DNA sequences
which encode the enzyme. The enzyme is useful in a
screening assay to identify compounds useful as immuno-
suppression agents.



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| (74) Agents: | BENNETT, Michael, Roy et al.; Russell McVeagh West-Walker, The Todd Building, Level 5, 171-177 Lambton Quay, Wellington 6001 (NZ). | Published | <i>With international search report.</i> |

(54) Title: ENZYME HAVING S-ADENOSYL-L-HOMOCYSTEINE HYDROLASE (AHCY) TYPE ACTIVITY

Alignment of DD4b5.3 AHCY-like domain with full-length AHCY amino acid sequences of human (hu), mouse (mu) and drosophila (dr).
Noted are conserved features shown to be important for AHCY function.

| |
|---|
| <pre> 45 150 160 170 180 190 200 210 220 230 240 4b TGRRSLRSISQSSTDYSSSAASYTDSQDVSPREKQQTNSKGSSNECKNKAQFGRRELEAEQDMSALISLRRAQGEPLAGAKIVGCNHLAQ hu MSDICLTVVVAIDIGIARAWGRQLDIAENEMPGILMMRERVGSYPLAGARLACCLHMTVZ mu MSDKLPLXKVADIGIARAWGRQLDIAENEMPGILMMRERVGSYPLAKLKGARLACCLHMTVZ dr MS. KPSYKVADISLAEWGAKAIIIAEENMPGIMACRKKYGSKPAAKGARYTGCLHMTVZ </pre> <pre> 250 260 270 280 290 300 310 320 330 340 4b IAVLTCETLGGIQRWSACHTYSTONEVATAKLEAGVAVIAGKESEDDEWGTDRCVNM DQWQANMIZDDGGDLTWWVYKTPNVFKICNGLVVEESV hu IAVLTCETLGGIQRWSACHTYSTONEVATAKLEAGVAVIAGKESEDDEWGTDRCVNM DQWQANMIZDDGGDLTWWVYKTPNVFKICNGLVVEESV mu IAVLTCETLGGIQRWSACHTYSTONEVATAKLEAGVAVIAGKESEDDEWGTDRCVNM DQWQANMIZDDGGDLTWWVYKTPNVFKICNGLVVEESV dr IAVLTCETLGGIQRWSACHTYSTONEVATAKLEAGVAVIAGKESEDDEWGTDRCVNM DQWQANMIZDDGGDLTWWVYKTPNVFKICNGLVVEESV </pre> <pre> ----- Cys residues involved in ----- ----- disulphide bonds ----- </pre> <pre> 350 360 370 380 390 400 410 420 430 440 4b AGVHRISIQLSKAGKICCPAIVVNDSVTKSKEQDNLYCRESLTDCIKRATDVAGKAVVAGYGDVGKCAQALRGFGARVLTEDPINAQAAAGCYS hu AGVHRISIQLSKAGKICCPAIVVNDSVTKSKEQDNLYCRESLTDCIKRATDVAGKAVVAGYGDVGKCAQALRGFGARVLTEDPINAQAAAGCYS mu AGVHRISIQLSKAGKICCPAIVVNDSVTKSKEQDNLYCRESLTDCIKRATDVAGKAVVAGYGDVGKCAQALRGFGARVLTEDPINAQAAAGCYS dr AGVHRISIQLSKAGKICCPAIVVNDSVTKSKEQDNLYCRESLTDCIKRATDVAGKAVVAGYGDVGKCAQALRGFGARVLTEDPINAQAAAGCYS </pre> <pre> ----- NAD+ co-factor bonding motif ----- </pre> <pre> 450 460 470 480 490 500 510 520 530 4b V.KLNVIROQDVVILQGKVVVTREHLDPMNSCIVCGAGHSNTEDIDTSIXTPSLTWERVRSDQDHVIWPDGKRVVLLAEGRLNLSCTV. ETVL hu VITMDAEGKAGLTVVTCGVDSEGRTECKMDAALCNIGHDVEIDVWNENAEVKVTKPQDVKRERKCHRTELLAEGRVWNECCMCHPSEVM mu VITMDAEGKAGLTVVTCGVDSEGRTECKMDAALCNIGHDVEIDVWNENAEVKVTKPQDVKRERKCHRTELLAEGRVWNECCMCHPSEVM dr VITMDAEGKAGLTVVTCGVDSEGRTECKMDAALCNIGHDVEIDVWNENAEVKVTKPQDVKRERKCHRTELLAEGRVWNECCMCHPSEVM </pre> <pre> 540 550 560 570 580 590 600 610 4b SITATDALAELVLYNAPEGRYKQDVYLPKCDEWYASLHEPSFDAPLTDTSQDQKQYLGKLNNGPFKPNNVRY hu SNSSETAQVTAQELWTHPD. KYPVGVHFLPKLDEAVAEAPLGLCNVKTAKLTERAOQYLGMPVINGPEPKPDHRY mu SNSSETAQVTAQELWTHPD. KYPVGVHFLPKLDEAVAEAPLGLCNVKTAKLTERAOQYLGMPVINGPEPKPDHRY dr SNSSETAQVTAQELWTHPD. KYPVGVHFLPKLDEAVAEAPLGLCNVKTAKLTERAOQYLGMPVINGPEPKPDHRY </pre> <p style="text-align: center;">^ Lys residue critical for enzyme function</p> |
|---|

(57) Abstract

The invention provides an enzyme having AHCY-type activity and DNA sequences which encode the enzyme. The enzyme has utility *inter alia* in a screening assay to identify compounds useful as immunosuppression agents.

INTERNATIONAL SEARCH REPORT

International Application No.
PCT/NZ 97/00133

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| A. CLASSIFICATION OF SUBJECT MATTER | | |
| Int Cl ⁶ : C12N 9/14, 15/55 | | |
| According to International Patent Classification (IPC) or to both national classification and IPC | | |
| B. FIELDS SEARCHED | | |
| Minimum documentation searched (classification system followed by classification symbols) See Electronic Database Box | | |
| Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched See Electronic Database Box | | |
| Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) Genebank, EMBL, Swissprot (Amino acids 177-314 of Fig.1) EKQQTNSKGSSNFCVKNIKQAEFGRREIEEQDMSALISLRKRAQGEK PLAGAKIVGCTHTAQTAQTVLIETLCALGAQCRWSACNTYSTQNEVAA ALAEAGVAVFAWKGESEDDFWWCIDRCVNMDGWQANMILDDG | | |
| C. DOCUMENTS CONSIDERED TO BE RELEVANT | | |
| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
| X | Ogawa, H. (et al) "Amino acid sequence of s-adenosyl-L-homocysteine hydrolase from rat liver". <u>Proc. Natl. Acad. Sci. USA</u> , vol.84, No.719, 1987, pages 719-723 see entire document | 1-18 (in part) |
| X | Martin, C.H. (et al) "Complete sequence of the bithorax complex of Drosophila". <u>Proc. Natl. Acad. Sci. USA</u> , vol.92, pp 8392-8402, 1995 see particularly Table 1 | 1-18 (in part) |
| <input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C | | <input type="checkbox"/> See patent family annex |
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| Date of the actual completion of the international search 11 December 1997 | Date of mailing of the international search report 16 DEC 1997 | |
| Name and mailing address of the ISA/AU AUSTRALIAN INDUSTRIAL PROPERTY ORGANISATION PO BOX 200 WODEN ACT 2606 AUSTRALIA Facsimile No.: (02) 6285 3929 | Authorized officer J.H. CHAN Telephone No.: (02) 6283 2340 | |

INTERNATIONAL SEARCH REPORT

International Application No.

PCT/NZ 97/00133

| C (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT | | |
|--|---|-----------------------|
| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
| X | Kashir, J. (et al) "Amino acid sequence of S-Adenosyl-L-Homocysteine Hydrolase from <u>DICTYOSTELIUM DISCOIDEUM</u> as deduced from the cDNA sequence", <u>Biochem. and Biophys. Res. Com.</u> , vol.153, no.1, pages 359-364. see entire document | 1-18 (in part) |
| X | GOMI, T. (et al) "S-Adenosyl homocysteinase from Rat Liver", <u>J. Biol. Chem.</u> , vol.261, no.29, pages 13422-13425, 1986. see entire document | 1-18 (in part) |



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(54) Title: ENZYME HAVING S-ADENOSYL-L-HOMOCYSTEINE HYDROLASE (AHCY) TYPE ACTIVITY

Alignment of DD455.3 AHCY-like domain with full-length AHCY amino acid sequences of human (hu), mouse (mu) and drosophila (dr). Noted are conserved features shown to be important for AHCY function.

(57) Abstract

The invention provides an enzyme having AHCY-type activity and DNA sequences which encode the enzyme. The enzyme has utility *inter alia* in a screening assay to identify compounds useful as immunosuppression agents.

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ENZYME HAVING S-ADENOSYL-L-HOMOCYSTEINE HYDROLASE (AHCY) TYPE ACTIVITY

This invention relates to a novel enzyme of the S-adenosyl-L-homocysteine hydrolase type and to methods which employ this enzyme.

5

BACKGROUND

- S-adenosyl-L-homocysteine hydrolase (AHCY) is an enzyme which is involved in the pathway of S-adenosylmethionine metabolism. S-adenosylmethionine (AdoMet) plays 10 a pivotal role as a methyl donor in a myriad of biological and biochemical events. For example, AdoMet donates methyl groups to small molecules like norepinephrine, yielding the potent product epinephrine; to medium sized molecules such as phosphatidylethanolamine, eventually forming phosphatidylcholine in the lipid bilayer; and to RNA and DNA, thereby modulating transcription and translation processes. 15 AdoMet - dependent methylation reactions have been postulated to regulate biological phenomena by post-translational modification as seen in the carboxymethylation of proteins and perhaps membrane functions via methylation of membrane phospholipids¹⁶.
- 20 S-adenosyl homocysteine (AdoHcy), formed after donation of the methyl group of AdoMet to a methyl acceptor is then hydrolysed to adenosine (Ado) and homocysteine (Hcy) by AdoHcy hydrolase (AHCY). Ado can either be deaminated by Ado deaminase (ADA) to inosine or be phosphorylated by Ado deaminase to become AMP.
- 25 Inhibition of AHCY results in an increase in intracellular levels of AdoHcy which is a potent inhibitor of AdoMet dependent methylation reactions²⁴. AdoHcy has also been reported to be a competitive inhibitor of PI Kinases⁵. PI kinase is required for PI metabolism and is involved in receptor mediated increases in intracellular calcium and the transduction of biochemical signals through cell membranes.
- 30 The applicants have now identified a novel enzyme of the AHCY type. It is towards this enzyme, DNA which encodes it, and methods which employ it that the present invention is *inter alia* broadly directed.

SUMMARY OF THE INVENTION

Therefore, in a first aspect, the present invention provides an enzyme having AHCY-type activity which includes amino acids 177 to 314 of the amino acid sequence of 5 Figure 1, or a functional portion or functional equivalent of said enzyme.

Conveniently, the enzyme comprises amino acids 183 to 614 or 1 to 614 of the amino acid sequence of Figure 1.

10 In a further aspect, the present invention provides a DNA sequence which encodes the AHCY-type enzyme above, said sequence selected from the group of:

- (a) a sequence which encodes an enzyme as defined above or a functional portion or equivalent thereof;
- 15 (b) a sequence which is a complement of a sequence (a);
- (c) a sequence which is a reverse complement of a sequence (a); and
- (d) a sequence which is a reverse sequence of a sequence (a).

Preferably, sequence (a) comprises nucleotides 529 to 945 of the Figure 1 sequence, 20 nucleotides 549 to 1844 of the Figure 1 sequence, or nucleotides 1 to 1844 of the Figure 1 sequence.

In yet a further aspect, the invention provides DNA constructs which comprise a DNA sequence as defined above.

25

In still a further aspect, the invention provides a method of modulating the activity of an AHCY-type enzyme as defined above in a patient which comprises administering to said patient a DNA construct as defined above.

30 In yet a further aspect, the invention provides a method of determining the modulatory potential of a test substance (particularly a compound), which method comprises the step of determining the ability of said substance to modulate the activity of the AHCY-type enzyme defined above.

Most conveniently, the modulatory potential which will be tested for will be the ability of the test substance to inhibit the AHCY-type enzyme defined above. In this way, immunosuppressant substances can be identified.

- 5 In still further aspects, the invention provides antibodies specific for the enzyme of the invention and optionally labelled nucleic acid probes (based upon the DNA sequence defined above). Such antibodies and probes have utility in the identification of abnormal HD cells in cytological preparations and tissue sections, and by *in situ* hybridisation, respectively.

10

DESCRIPTION OF THE DRAWINGS

- Although the present invention is broadly as described above, it will be appreciated that it also includes embodiments described below. In particular, a person skilled in 15 the art will appreciate that the invention will be better understood by reference to the accompanying drawing in which:

Figure 1 shows the cDNA and translated putative amino acid sequence of DD4b5.3. There is an open reading frame which extends without a stop codon for the full 5' 20 nucleotide sequence. The initiation codon has yet to be identified;

Figure 2 shows the alignment of DD4b5.3 AHCY domain with the full length AHCY amino acid sequences of human (hm), mouse (mu) and drosophila (dr). Some conserved features shown to be important for AHCY function are indicated;

25

Figure 3 provides a summary of the features of the DD4b5.3 sequence;

Figure 4 shows the results of RT-PCR analysis of DD4b5.3 mRNA expression in different leucocyte populations. A Southern blot of the products probed with a specific 30 internal probe is shown;

Figure 5 shows the results of RT-PCR analysis of different purified DC populations to examine their expression of DD4b5.3 mRNA. A semiquantitative analysis (ethidium stain) at different cycle number is shown;

Figure 6 shows the metabolic cycle of AdoMet and Ado Hcy; and

Figure 7 summarises the RAP-PCR protocol.

5 DESCRIPTION OF THE INVENTION

As a part of their general investigations into dendritic cells, the applicants have identified a gene encoding an enzyme of the AHCY type. The sequence of the identified gene has been found to have common features with the gene sequence of AHCY itself.

10

The applicants have also determined that this gene has a restricted expression pattern, being found in fresh blood dendritic cells and cultured blood dendritic cells, but not in T-cell or B-cell lymphocytes. It is therefore believed that the enzyme this gene encodes is substantially dendritic-cell-restricted. Due to the central role dendritic cells

15 play in initiation of the primary immune response to foreign antigen, manipulation of the activity of this enzyme within dendritic cells (especially inhibition) will have a direct effect on the immune response of the host. This represents the primary, although not sole, application of this invention.

20 In its first aspect, the invention therefore provides the enzyme itself. As indicated above, the enzyme of the invention has AHCY-type activity and includes amino acids 177 to 314 of the amino acid sequence of Figure 1; more preferably amino acids 183 to 614 of the amino acid sequence of Figure 1; and most preferably amino acids 1 to 614 of the amino acid sequence of Figure 1.

25

The invention also contemplates functional portions of the enzyme. As used herein, the "functional portion" of an enzyme is that portion which contains the active site essential for affecting the metabolic step, i.e. the portion of the molecule that is capable of binding one or more reactants or is capable of improving or regulating the rate of reaction. The active site may be made up of separate portions present on one or more polypeptide chains and will generally exhibit high substrate specificity.

The invention also includes functional equivalents of the enzyme described above.

The enzyme is a protein. A protein is considered a functional equivalent of another protein for a specific function if the equivalent protein is immunologically cross-reactive with, and has the same function as, the original protein. The equivalent may, for example, be a fragment of the protein, or a substitution, addition or deletion mutant 5 of the protein.

For example, it is possible to substitute amino acids in a sequence with equivalent amino acids using conventional techniques. Groups of amino acids known normally to be equivalent are:

10

- (a) Ala(A) Ser(S) Thr(T) Pro(P) Gly(G);
- (b) Asn(N) Asp(D) Glu(E) Gln(Q);
- (c) His(H) Arg(R) Lys(K);
- (d) Met(M) Leu(L) Ile(I) Val(V); and
- 15 (e) Phe(F) Tyr(Y) Trp(W).

Substitutions, additions and/or deletions in the enzyme may be made as long as the resulting equivalent enzyme is immunologically cross-reactive with, and has essentially the same function as, the native enzyme.

20

The equivalent enzymes will normally have substantially the same amino acid sequence as the native enzyme. An amino acid sequence that is substantially the same as another sequence, but that differs from the other sequence by means of one or more substitutions, additions and/or deletions is considered to be an equivalent sequence. 25 Preferably, less than 25%, more preferably less than 10%, and most preferably less than 5% of the number of amino acid residues in the amino acid sequence of the native enzyme are substituted for, added to, or deleted from.

The enzyme of the invention will most conveniently be produced using recombinant 30 techniques. Therefore, in a further embodiment, the present invention provides isolated complete or partial DNA sequences encoding, or partially encoding the enzyme of the invention. Specifically, the present invention provides isolated DNA sequences comprising nucleotides 529 to 945; 549 to 1844; or 1 to 1844 of the Figure 1 sequence. Complements of such isolated DNA sequences, reverse complements of

such isolated DNA sequences and reverse sequences of such isolated DNA sequences, together with variants of such sequences, are also provided. DNA sequences encompassed by the present invention include cDNA, genomic DNA, recombinant DNA and wholly or partially chemically synthesized DNA molecules.

5

The definition of the terms "complement", "reverse complement" and "reverse sequence", as used herein, is best illustrated by the following example. For the sequence 5' AGGACC 3', the complement, reverse complement and reverse sequence are as follows:

| | | |
|----|--------------------|---------------|
| 10 | complement | 3' TCCTGG 5' |
| | reverse complement | 3' GGTCCT 5' |
| | reverse sequence | 5' CCAGGA 3'. |

As used herein, the term "variant" covers any sequence which exhibits at least about 15 70% and, more preferably, at least about 90% identity to a sequence of the present invention. Most preferably, a "variant" is any sequence which has at least about a 99% probability of being the same as the inventive sequence. The probability for DNA sequences is measured by the computer algorithm FASTA (version 2.0u4, February 1996; Pearson W. R. et al., Proc. Natl. Acad. Sci., 85:2444-2448, 1988).

20

The DNA sequences may be isolated by high throughput sequencing of cDNA libraries. Alternatively, oligonucleotide probes based on the sequences provided in SEQ ID NOS. 4-6 can be synthesized and used to identify positive clones in either cDNA or genomic DNA libraries by means of hybridization or PCR techniques. Probes should be at least 25 about 10, preferably at least about 15 and most preferably at least about 20 nucleotides in length. Hybridization and PCR techniques suitable for use with such oligonucleotide probes are well known in the art. Positive clones may be analyzed by restriction enzyme digestion, DNA sequencing or the like.

30 In addition, the DNA sequences of the present invention may be generated by synthetic means using techniques well known in the art. Equipment for automated synthesis of oligonucleotides is commercially available from suppliers such as Perkin Elmer/Applied Biosystems Division (Foster City, CA) and may be operated according to the manufacturer's instructions.

The invention also provides DNA constructs. In one embodiment, the DNA constructs of the present invention include an open reading frame coding for at least a functional portion of the enzyme of the present invention.

- 5 For applications where amplification of enzyme activity is desired, the open reading frame is inserted in the DNA construct in a sense orientation, such that transformation with the DNA construct will lead to an increase in the number of copies of the gene and therefore an increase in the amount of enzyme. When down-regulation of activity is desired, the open reading frame is inserted in the DNA construct in an antisense
10 orientation, such that the RNA produced by transcription of the DNA sequence is complementary to the endogenous mRNA sequence. This, in turn, will result in a decrease in the number of copies of the gene and therefore a decrease in the amount of enzyme. Alternatively, regulation can be achieved by inserting appropriate sequences or subsequences (e.g. DNA or RNA) in ribozyme constructs.

15

- In a second embodiment, the DNA constructs comprise a nucleotide sequence including a non-coding region of a gene coding for the enzyme of the present invention, or a nucleotide sequence complementary to such a non-coding region. As used herein the term "non-coding region" includes both transcribed sequences which are not
20 translated, and non-transcribed sequences within about 2000 base pairs 5' or 3' of the translated sequences or open reading frames. Examples of non-coding regions which may be usefully employed in the inventive constructs include introns and 5'-non-coding leader sequences. Transformation with such a DNA construct may lead to a reduction in the amount of enzyme synthesized by the process of cosuppression.

25

- The DNA constructs of the present invention further comprise a gene promoter sequence and a gene termination sequence, operably linked to the DNA sequence to be transcribed, which control expression of the gene. The gene promoter sequence is generally positioned at the 5' end of the DNA sequence to be transcribed, and is
30 employed to initiate transcription of the DNA sequence. Gene promoter sequences are generally found in the 5' non-coding region of a gene but they may exist in introns (Luehrs, K. R., *Mol. Gen. Genet.* 225:81-93, 1991) or in the coding region. When the construct includes an open reading frame in a sense orientation, the gene promoter sequence also initiates translation of the open reading frame. For DNA constructs

comprising either an open reading frame in an antisense orientation or a non-coding-region, the gene promoter sequence consists only of a transcription initiation site having a RNA polymerase binding site.

- 5 A variety of gene promoter sequences which may be usefully employed in the DNA constructs of the present invention are well known in the art. The promoter gene sequence, and also the gene termination sequence, may be endogenous or may be exogenous, provided the promoter is functional in the host. Preferably, gene promoter and termination sequences are from the inventive sequences themselves.

10

The gene termination sequence, which is located 3' to the DNA sequence to be transcribed, may come from the same gene as the gene promoter sequence or may be from a different gene. Many gene termination sequences known in the art may be usefully employed in the present invention. However, preferred gene terminator

15 sequences are those from the original enzyme gene.

Techniques for operatively linking the components of the DNA constructs are well known in the art and include the use of synthetic linkers containing one or more restriction endonuclease sites as described, for example, by Maniatis et al., (*Molecular*

- 20 *Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratories, Cold Spring Harbor, NY, 1989). The DNA construct of the present invention may be linked to a vector having at least one replication system, for example, *E. coli*, whereby after each manipulation, the resulting construct can be cloned and sequenced and the correctness of the manipulation determined.

25

Having described the enzyme and the DNA constructs the present invention will now be illustrated by reference to the following non-limiting examples.

Example 1

- 30 (a) Identification of enzyme-encoding gene

The Differential Display (DD) technique RAP-PCR was employed, using Hodgkins cell line L428 and monocyteid cell line U937. The RAP-PCR protocol is as shown in Figure 7, and was performed as follows:

- Total RNA extracted from L428 and U937 cell lines (Step 1) was used for first stand cDNA was synthesis using an arbitrary oligonucleotide primer (Step 2). An aliquot of the RT reaction was used in a subsequent PCR with the same arbitrary primer in the presence of 32P-labelled dCTP. The first five cycles of the PCR was performed at the 5 low annealing temperature of 40oC (low stringency) to allow mismatches between the arbitrary primer and cDNA template (Step 3), followed by 30 cycles at 60oC annealing temperature (high stringency) to amplify specifically the products which have incorporated the arbitrary primer (Step 4). The PCR products were electrophoresed non-denaturing sequencing gels and subjected to autoradiography. PCR products on 10 the L428 lanes but not U937 lanes were excised from the gel using the autoradiogram as a guide (Step 5), and used as template to reamplify the products with the arbitrary primer (Step 6). Reamplified L428 specific bands were cloned in pBluescript and DNA sequenced.
- 15 One clone, called DD4b.5.3, contained a 416 bp fragment encoding part of the enzyme of the invention.

(b) **Isolation of a full length cDNA for DD4b5.3**

- The DD4b5.3 cDNA fragment was used to probe a Lambda ZAP express (Stratagene) 20 L428 cDNA library produced in this laboratory. A near full length clone of 2.2 kb was identified (6.1222(1)) which was sequenced using a LI-COR automated sequencer (Figure 1, nucleotides 241-2563).

- Rapid amplification of cDNA ends (RACE) was used to identify further 5' cDNA 25 sequence using L428 cDNA as a template. RACE products were cloned and sequenced revealing further 5' sequence. Subsequently a 2nd cDNA clone (2.11(1)b) was isolated from the L-428 library. This provided further 5' sequence (Figure 1; nucleotides 1-240) with two bases of identical overlapping sequence with the first clone.
- 30 Comparison of the nucleic acid sequence revealed a significant portion of identity with the cDNA sequence for an enzyme called L-adenosine-S homocysteine hydrolase (AHCY).

Translation of the cDNA sequence for DD4b5.3 revealed an open reading frame as indicated in Figure 1. Comparison of the putative DD4b5.3 amino acid sequence with the AHCY sequences of human¹, mouse (Genbank Accession no. L32836) and drosophila (Genbank Accession no. X95636) revealed extensive similarity to the AHCY sequences. (Figure 2). A further analysis of the structure is shown in Figure 3.

DD4b5.3 has a similar enzymatic function to AHCY based on significant identities in the AHCY related sequence. Thus DD4b5.3 has:

- 10 i) Cys¹¹³ and Cys¹⁹⁵ are conserved (numbering based on AHCY amino acid sequence (DD4b5.3 sequence)). Modification of these affects enzymatic function¹³. In contrast Cys⁴²¹ is non essential¹³ and this is substituted (Lys) in DD4b5.3.
- 15 ii) Lys⁴²⁶ identified as critical for AHCY function is preserved in DD4b5.3¹⁴. (Figure 2)
- 20 iii) Val¹⁷⁵ and Lys¹⁸⁶ predicted to be involved in Ade ring binding are conserved in DD4b5.3¹⁵. In contrast, Val³¹⁹ and Arg³²⁷ also predicted to be involved¹⁵ are not but the QVD sequence between them is, suggesting this may allow for an alternative specificity.
- 25 iv) Glu¹⁹⁷¹⁶ and Serine¹⁹⁸ which are also related to the active enzyme site are conserved in DD4b5.3.
- v) The cofactor NAD binding site¹⁷ is highly conserved (Lys²¹⁴ - Asp²³⁵) and the critical G-G--G binding site is present in DD4b5.3. (Figure 2)
- 30 vi) Eight Cys residues are thought to be involved in disulphide bonding. These contribute to maintaining the globular structure of the 4 subunits (~47,000) which make up the tetramer (~190,000) AHCY molecule in mammals. Seven of eight are preserved in DD4b5.3 amino acid sequence.

Example 2Tissue DistributionExperimental 1Methods5 *Northern blotting*

Total RNA was extracted from L428 and U937 cell lines, electrophoresed on formaldehyde-denaturing agarose gels, transferred to nylon membranes and probed with ^{32}P labeled DD clones.

10 *RT-PCR analysis*

Based on sequence data, clone-specific oligonucleotide primers were synthesised to assess tissue distribution by RT-PCR. Two panels of haemopoietic cell populations were screened:

- 1) Cultured cell lines. Total RNA was extracted from cell lines, treated with RNase-free DNase I, and used for first stand cDNA synthesis. Aliquots of cDNA were used in PCRs with specific oligonucleotide primers.
- 2) Freshly isolated cells. First strand cDNA synthesis was performed directly on whole-cells isolated from human peripheral blood, based on established methods. Template consisted of 500 purified cells and the entire reaction was used in the subsequent PCR.

RT-PCR products were assessed by agarose gel electrophoresis. Identity of the products was confirmed by internal oligonucleotide hybridisation.

25 **Results***Northern blot analysis*

Northern blot analysis reveals DD4b.5.3 probe hybridises only to the L428 lanes.

RT-PCR analysis of cell lines

- 30 Because L428 is a Hodgkin's disease cell line it is possible that DD products isolated from L428 may be expressed in other Hodgkin's cell lines. We assessed the expression of clone DD4b.5.3 in a cell line panel by RT-PCR (Table 1). The results indicate clone DD4b.5.3 is expressed showing a different pattern of expression across the different cell lines.

Table 1

| | |
|-------------|---------|
| Cell line | DD4b.53 |
| L428 | +++ |
| act.U937 | + |
| U937 | + |
| Monomac | + |
| THP1 | ++ |
| NB4 | + |
| KG1 | - |
| HDLM2 | +++ |
| KMH2 | ++ |
| Raji | ++ |
| Mann | ++ |
| EBV-B cells | + |
| Jurkat | ++ |
| K562 | - |

Relative band intensities: +++ strong, ++ moderate, + weak, - no band visible.

20 β2-microglobulin cDNA control PCR also performed.

RT-PCR analysis of fresh cells

To assay the expression of the differential display clones in normal cell populations, RT-PCR was performed on freshly isolated cells. The results are summarised in Table
25 2.

Table 2
Summary of RT-PCR results

| RT-PCR Template | Phenotype | DD4b.5.3 |
|-----------------|------------------------------|---------------------------------------|
| 5 | Fresh blood DC | DR ⁺ , Mix |
| | Cultured Blood DC | DR ⁺ , CMRF44 ⁺ |
| | Fresh Blood Monocytes | CD14 ⁺ |
| | Cultured Blood Monocytes | CD14 ⁺ |
| 10 | Fresh B Blood Lymphocytes | CD19 ⁺ |
| | Cultured B Blood Lymphocytes | CD19 ⁺ |
| | Blood T Lymphocytes | CD3 ⁺ |
| | NK cells | CD16 ⁺ , CD57 ⁺ |

1 Semi-Nested PCR

- 15 Relative band intensities compared by ethidium bromide staining:
 ++ moderate, + weak, +/- very weak, - no product visible.

Experimental 2

- i) Cell lines
 20 Northern blot analysis using RNA obtained from cell lines revealed specific hybridisation to a L428 transcript which was not detected in U937 (data not shown)

- ii) Leucocyte Populations
 Semi-quantitative RT-PCR was performed on cDNA template derived from normal leukocyte cell populations using specific oligonucleotide primers. The cDNA equivalent from 125 cells was used in each reaction (Figure 4). To confirm the specificity of the DD4b5.3 RT-PCR (nucleotides 529-944), the products (416 bp) were blotted onto nylon membrane and probed with an internal digoxigenin (Boehringer Manheim) labelled oligonucleotide. Control β 2-microglobulin RT-PCR analysis confirmed the 30 template integrity.

Abundant DD4b5.3 message was observed after 1 min exposure, in activated DC (lane 2), and fresh DC (lane 1). Minimal expression in some other leukocyte populations was noted, but only after prolonged 60 min exposure.

5 iii) DC Lineage

RT-PCR was performed using cDNA template from a range of different DC populations representing a putative DC differentiation/ activation pathway. (Figure 5). Using a range of cycle numbers an estimate of DD4b5.3 mRNA context in each type of DC preparation was obtained. Increased levels of DD4b5.3 mRNA were associated with
10 the differentiation/ activation of DC derived from the blood and non-lymphoid tissues but little DD4b5.3 mRNA was detected in "mature" tonsil DC.

Conclusion

The enzyme of the invention has a dendritic-cell-restricted expression pattern.

15

INDUSTRIAL APPLICATION

The significant amino acid identity of DD4b5.3 with human AHCY suggests that it has a related enzymatic function (ie. is of the AHCY-type) (see enzyme 4 in Figure 6).

20 As turnover of (AdoMet) varies between tissues it seems probable that different cell types may express different AHCY like enzymes for the pathways illustrated in Figure 6 or other pathways. DD4b5.3 may have such a function in DC. Its relatively selective expression in DC and its upregulation at certain stages of DC differentiation/ activation suggest it may be critical to DC function.

25

The novel N terminal sequence of the enzyme may be involved in localising DD4b5.3 to a specific intracellular compartment or play a part in regulating its enzymatic function perhaps by acting as a regulatory domain and binding to other proteins.

30 The enzyme of the invention will therefore have a number of potential applications. These include in immunomodulation (ie. manipulation of the immune response of a host). The enzyme will also have application in a screening programme to identify substances (usually compounds) which have either a stimulatory or inhibitory effect on its activity. More preferably, the program will be directed to determining the

immunosuppressive potential of a test compound with reference to the ability of the compound to inhibit enzyme activity.

In this method, procedures identical or analogous to those outlined in Wolos *et al.*,
5 *Journal of Immunology* (1993), *supra* can be employed. Other conventional procedures for screening the effect of a test compound on enzyme activity can also be used.

Compounds identified in this way will have numerous potential applications.

10

AHCY itself can be inhibited by many nucleosides^{2,6-9}. The compound MDL 28,842 has been reported to be immunosuppressive. It inhibited Con A and PWM induced proliferation of human peripheral blood mononuclear cells¹⁰. MDL 28,842 inhibited the Con A induced mouse T lymphocyte proliferation but not LPS induced B cell
15 proliferation and reduced T lymphocyte IL-2 and IL-2R production¹⁰. *In vivo*, MDL 28,842 inhibited the mouse antibody response to the T lymphocyte dependent antigen ovalbumin¹⁰. The same compound prevented collagen induced arthritis in mice¹¹. Administration at 5mg/kg/day ip for 6 days prolonged skin graft survival to 12.2 days compared to 8.7 days in controls, a superior result to that obtained with Cyclosporin
20 A at 5mg/kg/day¹². It may also inhibit heart allograft rejection in rats¹².

Equivalent applications are likely for compounds which inhibit the enzyme of the invention.

25 Blocking or inhibiting the activity of enzyme DD4b5.3 also gives rise to the following potential applications:

- Inhibition of DD4b5.3 may block DC function (eg: migration, antigen uptake, antigen processing and presentation or costimulation of T lymphocytes). This
30 may be profoundly immunosuppressive. DD4b5.3 can be expressed as a protein¹⁷ and nucleoside analogues or other compounds designed which inhibit its function. A precedent for selective functional inhibition is illustrated by the use of the AHCY inhibitory compound C3 ado which inhibits macrophage IL-1 synthesis but not T lymphocyte proliferation¹⁸.

The identification of safe agents for *in vivo* use (note similar compounds have been given to animals without major side-effects), which have a selective inhibitory effect on DC may allow the design of an important new immunosuppressive drug. This would have applications in all forms of
5 transplantation and auto-immune disease.

- It is (theoretically) possible that blocking DD4b5.3 enzymatic function may have the reverse function, ie: boost their functional capabilities. As such, DD4b5.3 compounds might have immunostimulatory properties for vaccination eg.
10 against infectious disease or for the immunotherapy of cancer.
 - It is also conceivable that blocking DD4b5.3 might modify DC growth and differentiation facilitating the collection of DC for therapeutic use.
- 15 The enzyme of the invention can also be used to generate antibodies for example by the method of Kohler and Milstein (Kohler, G. and Milstein, C., Nature 256 495-497 (1975). Due to the restricted expression of the enzyme of the invention, such antibodies will have potential utility in the identification of DC in cytological preparations and tissue sections. The abnormal cells in Hodgkins disease have many
20 features similar to DC. The enzyme is expressed in HD cell lines and the identification of the abnormal cells (Reed Sternberg cells and the related Hodgkins cells) which express the enzyme will be of value in the diagnosis of disorders such as Hodgkins disease²⁰.
- 25 The DNA sequence of the invention or any part thereof can also be used as a probe to discriminate for abnormal HD cells by *in situ* hybridisation. The use of nucleic acid probes is standard, for example as described in Maniatis et al., "Molecular Cloning: A Laboratory Manual", Cold Spring Harbour (1982).
- 30 Other applications include the following:
- The DD4b5.3 sequence can be used to design anti -sense (anti-mRNA) reagents to suppress DC function, achieving the possible outcomes described above.

- The novel amino acid sequence towards the N terminus of the AHCY-like sequence may be used to target other compounds into the appropriate intracellular site to modify molecular functions, eg: to target other inhibitors of DD4b5.3.

5

- The identification of a novel AHCY-like sequence with additional N terminal sequence suggests there may be an extended family of such AHCY-like enzymes. The cDNA sequence of DD4b5.3 could be used to screen for other members of this family by a range of standard molecular biology techniques.

10

Those persons skilled in the art will of course appreciate that the above description is provided by way of example only and that the invention is not limited thereto.

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CLAIMS

1. An enzyme having AHCY-type activity which includes amino acids 177 to 314 of the amino acid sequence of Figure 1, or a functional portion or functional equivalent of said enzyme.
5
2. An enzyme according to claim 1 which comprises amino acids 183 to 614 of the amino acid sequence of Figure 1.
3. An enzyme according to claim 1 which comprises amino acids 1 to 614 of the
10 amino acid sequence of Figure 1.
4. An isolated DNA sequence comprising a nucleotide sequence selected from the group consisting of:
 - (a) a sequence which encodes an enzyme according to claim 1 or a functional portion or equivalent thereof;
 - (b) a sequence which is a complement of a sequence (a);
 - (c) a sequence which is a reverse complement of a sequence (a); and
 - (d) a sequence which is a reverse sequence of a sequence (a).
- 20 5. A DNA sequence according to claim 4 in which sequence (a) comprises nucleotides 529 to 945 of the Figure 1 sequence.
6. A DNA sequence according to claim 4 in which sequence (a) comprises nucleotides 549 to 1844 of the Figure 1 sequence.
- 25 7. A DNA sequence according to claim 4 in which sequence (a) comprises nucleotides 1 to 1844 of the Figure 1 sequence.
8. A DNA construct comprising a DNA sequence according to any one of claims 4
30 to 7.
9. A DNA construct comprising, in the 5'-3' direction,:
 - (a) a gene promoter sequence;

- (b) an open reading frame coding for at least a functional portion of an enzyme according to claim 1; and
 - (c) a gene termination sequence.
- 5 10. A DNA construct according to claim 9 wherein the open reading frame is in a sense orientation.
11. A DNA construct according to claim 9 wherein the open reading frame is in an anti-sense orientation.
- 10 12. A DNA construct comprising, in the 5'-3' direction,:
 - (a) a gene promoter sequence;
 - (b) a non-coding region of a gene coding for an enzyme according to claim 1; and
 - (c) a gene termination sequence.
- 15 13. A method for modulating the activity of an enzyme according to claim 1 in a patient, comprising administering to said patient a DNA construct according to any one of claims 8 to 12.
- 20 14. A method of amplifying the activity of an enzyme according to claim 1 in a patient comprising administering to said patient a DNA construct according to claim 10.
- 25 15. A method of supressing the activity of an enzyme according to claim 1 in a patient comprising administering to said patient a DNA construct according to claim 11 or claim 12.
- 30 16. A method of determining the modulatory potential of a compound on an enzyme according to claim 1 which comprises the step of determining the ability of said compound to modulate the activity of said enzyme.
17. An antibody which binds an enzyme according to claim 1.

18. An optionally labelled nucleic acid probe capable of hybridising, under high stringency, to a nucleotide sequence of Figure 1.

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DD4b5.3 Coding Sequence

Length 2563 bp

1 GGCAGCGGGCAGGTCGGAGCTCGGAGCTGCTGCTTCTGGTTCTTGTGGCCACCGTCGCT
 1 R G Q V G A R S C C F W F S C G H R R C -
 60
 61 GTCCGGCTGCCTTGGCTGCCAACAGACAAGGGTGGGCCACAGCACCTCAGAACCGA
 61 P A A L G C R T D K A W A T A P Q K P T -
 120
 121 CGCAGCTCGACGCAGGGGCCGGCAGGAGGGTGGCGATCGCGTGTGGAGGGCGCCGC
 121 Q L D A G A G R R V G D R V S E G A A R -
 180
 181 GGGCAGGCGGGCGGGCGCCAGAGGGAAAGAGGCGGGGGCGGGTCAGCCGCTGGCC
 181 A G G R A P E G E R G G G G S A A A G R -
 240
 241 GGGCCGGCGGGGAATGTCGATGCCTGACGCGATGCCGCTGCCGGGTGGGGAGGAGC
 241 A G G G M S M P D A M P L P G V G E E L -
 300
 301 TGAAGCAGGCCAAGGAGATCGAGGACGCCAGAGACTCCTCATGGCCACCGTCACCA
 301 K Q A K E I E D A E K Y S F M A T V T K -
 360
 361 AGGCGCCAAGAACAAATCCAGTTGCTGATGACATGCAGGAGTTACCAAATTCCCCA
 361 A P K K Q I Q F A D D M Q E F T K F P T -
 420
 421 CAAAAACTGGCGAAGATCTTGTCTCGCTCGATCTCACAGTCCTCCACTGACAGCTACA
 421 K T G R R S L S R S I S Q S S T D S Y S -
 480
 481 GTTCAGCTGCATCCTACACAGATACTGATGAGGTTCTCCCCGAGAGAACAGCAGC
 481 S A A S Y T D S S D D E V S P R E K Q Q -
 540
 541 AAACCAACTCCAAGGGCAGCAGCAATTCTGTGTGAAGAACATCAAGCAGGAGAACATTG
 541 T N S K G S S N F C V K N I K Q A E F G -
 600
 601 GACGCCGGAGATTGAGATTGAGACACTCTGCTGCTCTGATTCACAGGAAAC
 601 R R E I E I A E Q D M S A L I S L R K R -
 660
 661 GTGCTCAGGGGAGAACGCCCTGGCTGGTGTAAAATAGTGGCTGTACACACATCACAG
 661 A Q G E K P L A G A K I V G C T H I T A -
 720
 721 CCCAGACAGCGGTGTTGAGACACTCTGCTGCCCTGGGGCTCAGTGCCGCTGGCTG
 721 Q T A V L I E T L C A L G A Q C R W S A -
 780
 781 CTTGTAACATCTACTCAACTCAGAATGAAAGTAGCTGCAGCACTGGCTGAGGCTGGAGTTG
 781 C N I Y S T Q N E V A A A L A E A G V A -
 840
 841 CAGTGTGCTTGGAAAGGGCGAGTCAGAACAGATGACTCTGGTGGTATTGACCGCTGTG
 841 V F A W K G E S E D D F W W C I D R C V -
 900
 901 TGAACATGGATGGGTGGCAGGCCAACATGATCCTGGATGATGGGGAGACTAACCACT
 901 N M D G W Q A N M I L D D G G D L T H W -

FIG. 1

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GGGTTTATAAGAAGTATCCAAACGTGTTAAGAAGATCCGAGGCATTGTGGAAGAGAGCG
 961 -----+-----+-----+-----+-----+-----+ 1020
 V Y K K Y P N V F K K I R G I V E E S V -

 TGACTGGTGTTCACAGGCTGTATCAGCTCTCCAAAGCTGGGAAGCTCTGTGTTCCGGCCA
 1021 -----+-----+-----+-----+-----+-----+ 1080
 T G V H R L Y Q L S K A G K L C V P A M -

 TGAACGTCAATGATTCTGTTACCAAACAGAAGTTGATAACTTGTACTGCTGCCGAGAAC
 1081 -----+-----+-----+-----+-----+-----+ 1140
 N V N D S V T K Q K F D N L Y C C R E S -

 CCATTTGGATGCCCTGAAGAGGACCACAGATGTGATGTTGGGGAAACAAGTGGTGG
 1141 -----+-----+-----+-----+-----+-----+ 1200
 I L D G L K R T T D V M F G G K Q V V V -

 TGTGTGGCTATGGTAGGTAAGGCAAGGGCTGCTGTGCTCTCAAAGCTCTGGAGCAA
 1201 -----+-----+-----+-----+-----+-----+ 1260
 C G Y G E V G K G C C A A L K A L G A I -

 TTGTCACATTACCGAAATCGACCCCCATCTGTGCTCTGCAGGCCATGGATGGTTCA
 1261 -----+-----+-----+-----+-----+-----+ 1320
 V Y I T E I D P I C A L Q A C M D G F R -

 GGGTGGTAAAGCTAAATGAAGTCATCCGGCAAGTCGATGTCGAATAACTGCACAGGAA
 1321 -----+-----+-----+-----+-----+-----+ 1380
 V V K L N E V I R Q V D V V I T C T G N -

 ATAAGAATGTAGTGACACGGGAGCACTGGATCGCATAAAAACAGTTGTATCGTATGCA
 1381 -----+-----+-----+-----+-----+-----+ 1440
 K N V V T R E H L D R M K N S C I V C N -

 ATATGGGCCACTCCAACACAGAAATCGATGTGACCAGCCTCCGACTCCGGAGCTGACGT
 1441 -----+-----+-----+-----+-----+-----+ 1500
 M G H S N T E I D V T S L R T P E L T W -

 GGGAGCGAGTACGTTCTCAGGTGGACCATGTCATCTGGCCAGATGGCAAACGAGTTGTCC
 1501 -----+-----+-----+-----+-----+-----+ 1560
 E R V R S Q V D H V I W P D G K R V V L -

 TCCTGGCAGAGGGCGTACTCAATTGAGCTGCTCCACAGTTCCCACCTTGTCTGT
 1561 -----+-----+-----+-----+-----+-----+ 1620
 L A E G R L L N L S C S T V P T F V L S -

 CCATCACGCCACACAGGCTTGGCACTGATAGAACTCTATAATGCACCCGAGGGGC
 1621 -----+-----+-----+-----+-----+-----+ 1680
 I T A T T Q A L A L I E L Y N A P E G R -

 GATACAAGCAGGATGTACTTGCTTCTTAAGAAAATGGATGAATACGTTGCCAGCTTGC
 1681 -----+-----+-----+-----+-----+-----+ 1740
 Y K Q D V Y L L P K K M D E Y V A S L H -

 ATCTGCCATCATTGATGCCACCTTACAGAGCTGACAGATGACCAAGCAAAATCTGG
 1741 -----+-----+-----+-----+-----+-----+ 1800
 L P S F D A H L T E L T D D Q A K Y L G -

 GACTCAACAAAATGGCCATTCAAACCTAATTATTACAGATACTAATGGACCATACTAC
 1801 -----+-----+-----+-----+-----+-----+ 1860
 L N K N G P F K P N Y Y R Y *

 CAAGGACCAGTCCACCTGAACCACACACTCTAAAGAAATATTTTAAGATAACTTTAT
 1861 -----+-----+-----+-----+-----+-----+ 1920

 TTTCTTCTTACTCCTTCTTGTATTTCTTCTATAATTCATTCTGTGTTTCATC
 1921 -----+-----+-----+-----+-----+-----+ 1980

 TCATTATCCAAGTTCTGCAGACCACACAGGAACCTGCTCATGGCTCTTAGATGAAATA
 1981 -----+-----+-----+-----+-----+-----+ 2040

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GAAGTTCAGGGTCCCTCACTCTAGTCACTAAAGAAGGATTTACTCCCCAGCCCAGAAA
2041 -----+-----+-----+-----+-----+-----+ 2100

GGTGATTCTTCTCTTACCATTTCTGGGGACTTAGTCTTAATTAGGTACCTTATTAACA
2101 -----+-----+-----+-----+-----+-----+ 2160

GGAAATGCTAAGGTACCTCTGTGGAACAATCTGCAATGTCTAAATCGCCTAAAAGA
2161 -----+-----+-----+-----+-----+-----+ 2220

GCCCATTCTTAGCTGCTGAAATCAGTGCTTTCACTCTTCAGAGAACAGGATGGT
2221 -----+-----+-----+-----+-----+-----+ 2280

ACCTACCCGGCAGGTAGGTTAGATGTGGGTGGTCATGTTAATTCCCTAGAAGTTCCA
2281 -----+-----+-----+-----+-----+-----+ 2340

AGCCCTTTCTGCGTAAAGGTGGTATGTCCAGTCAGAGATGTGTATAATGAGCATGG
2341 -----+-----+-----+-----+-----+-----+ 2400

CTTGTAAAGATCAGGAGGCCACTGGATTATAGTATAGCCCTCCTCCACTCCCACCA
2401 -----+-----+-----+-----+-----+-----+ 2460

GACTTGCTCATTTTCGAGTTAACTAGACTACACTCTATTGAGTTAATTTGTCC
2461 -----+-----+-----+-----+-----+-----+ 2520

TCTAGGATTATTCTGTTGTCCAAAAAAAAAAAAAAA
2521 -----+-----+-----+-----+--- 2563

FIG 1 (Cont)

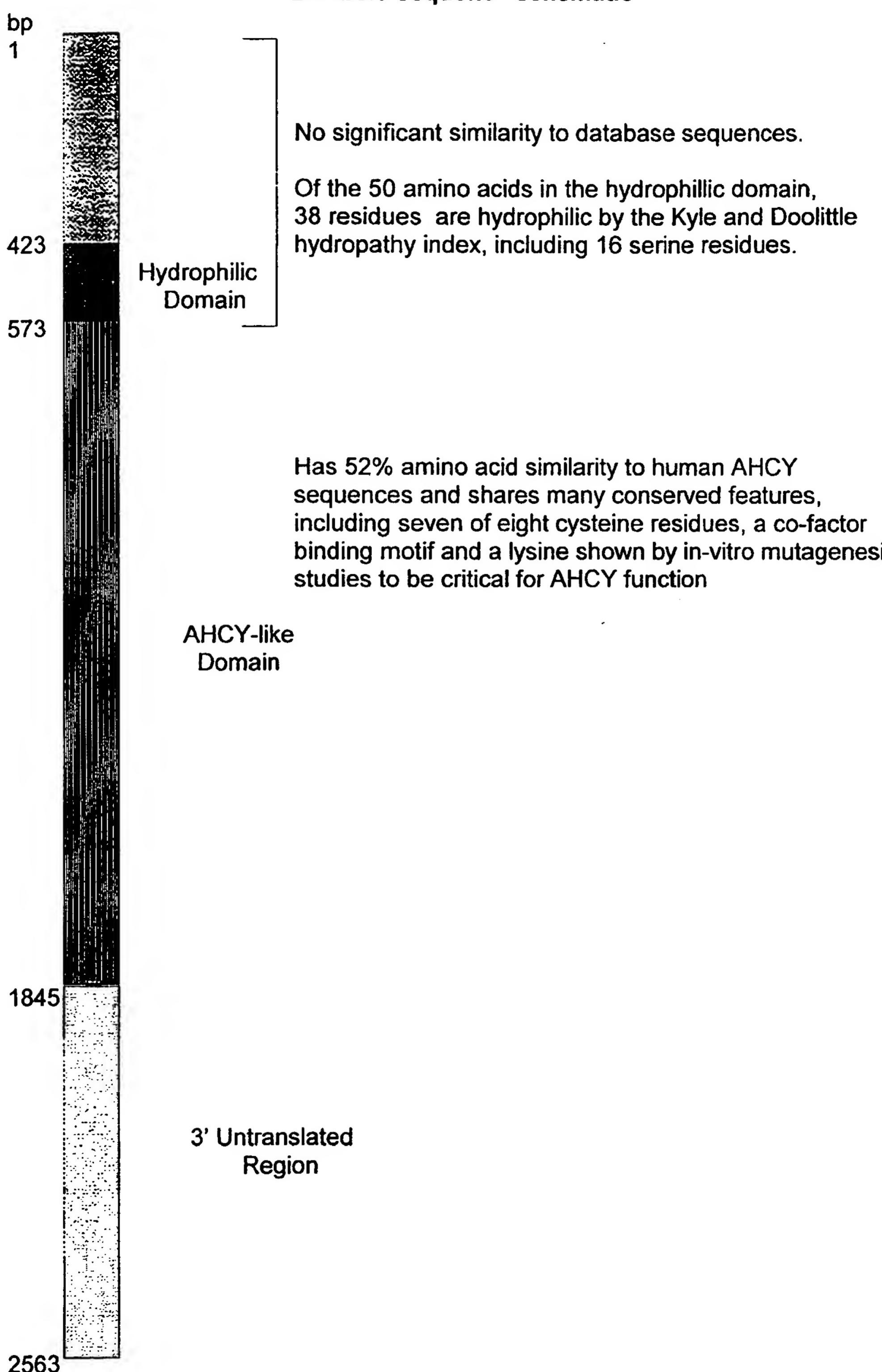
Alignment of DD4b5.3 AHCY-like domain with full-length AHCY amino acid sequences of human (hu), mouse (mu) and drosophila (dr).
Noted are conserved features shown to be important for AHCY function.



FIG 2

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DD4b5.3 sequence schematic



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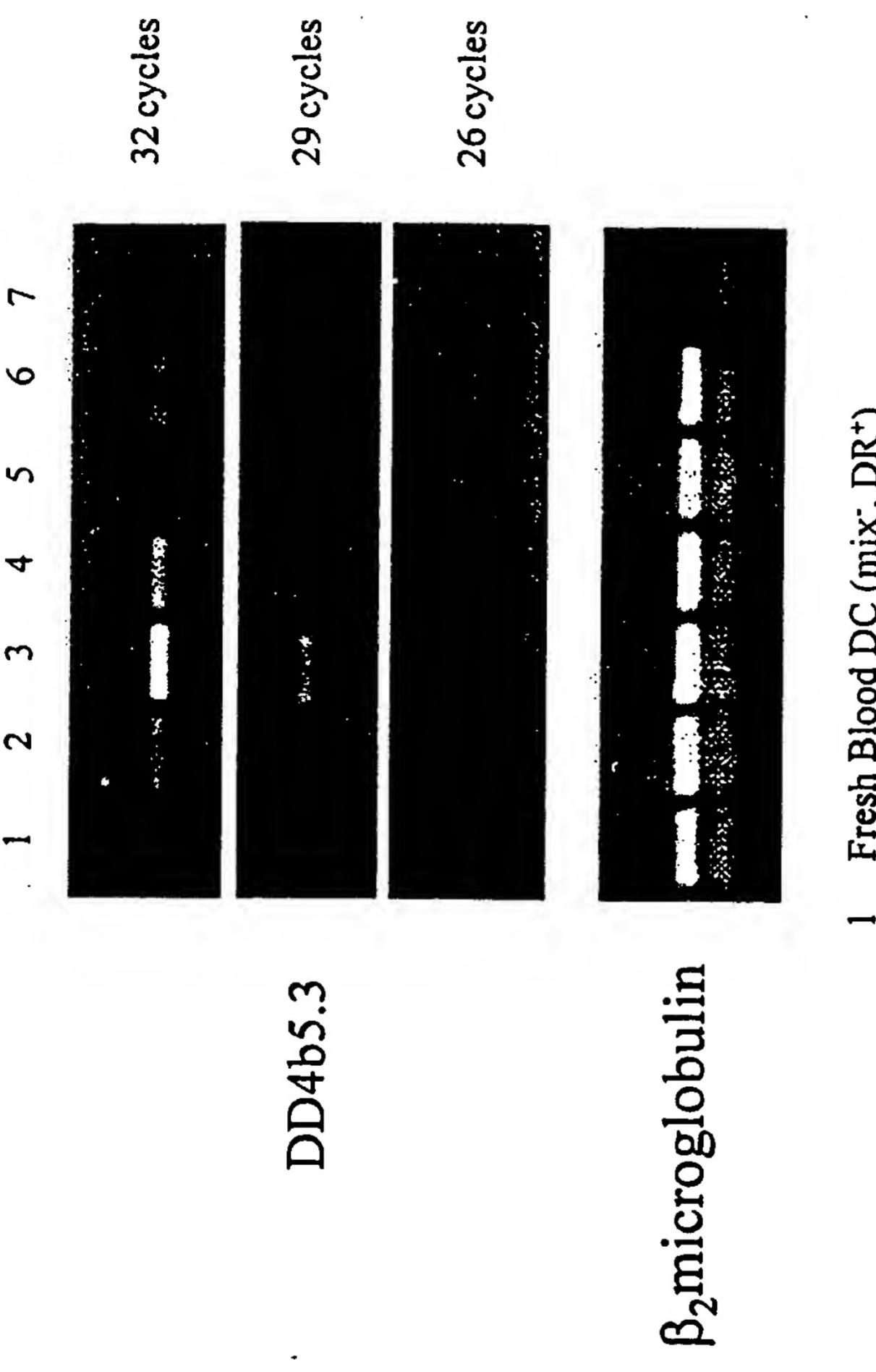
Southern blot analysis of DD4b5.3 RT-PCR results

Fresh DC (Mix⁻, DR⁺)Cultured DC (CMRF44⁺)Monocytes (CD14⁺)Cultured Monocytes (CD14⁺)Blymphocytes (CD19⁺)Cultured B Lymphocytes (CD19⁺)T Lymphocytes (CD3⁺)NK Cells (CD16⁺, CD57⁺)1 minute
exposure60 minutes
exposure

FIG 4

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Expression of DD4b5.3 in DC lineage panel, as assessed by RT-PCR



- 1 Fresh Blood DC (mix; DR⁺)
- 2 Cultured blood DC (CMRF-44⁺)
- 3 Langerhans cells (migratory, CMRF-44⁺)
- 4 Dermal DC (CMRF-44⁺)
- 5 Tonsil cells, mix; CMRF-44⁺
- 6 Tonsil cells, mix, CMRF-44⁻
- 7 H₂O control

FIG 5

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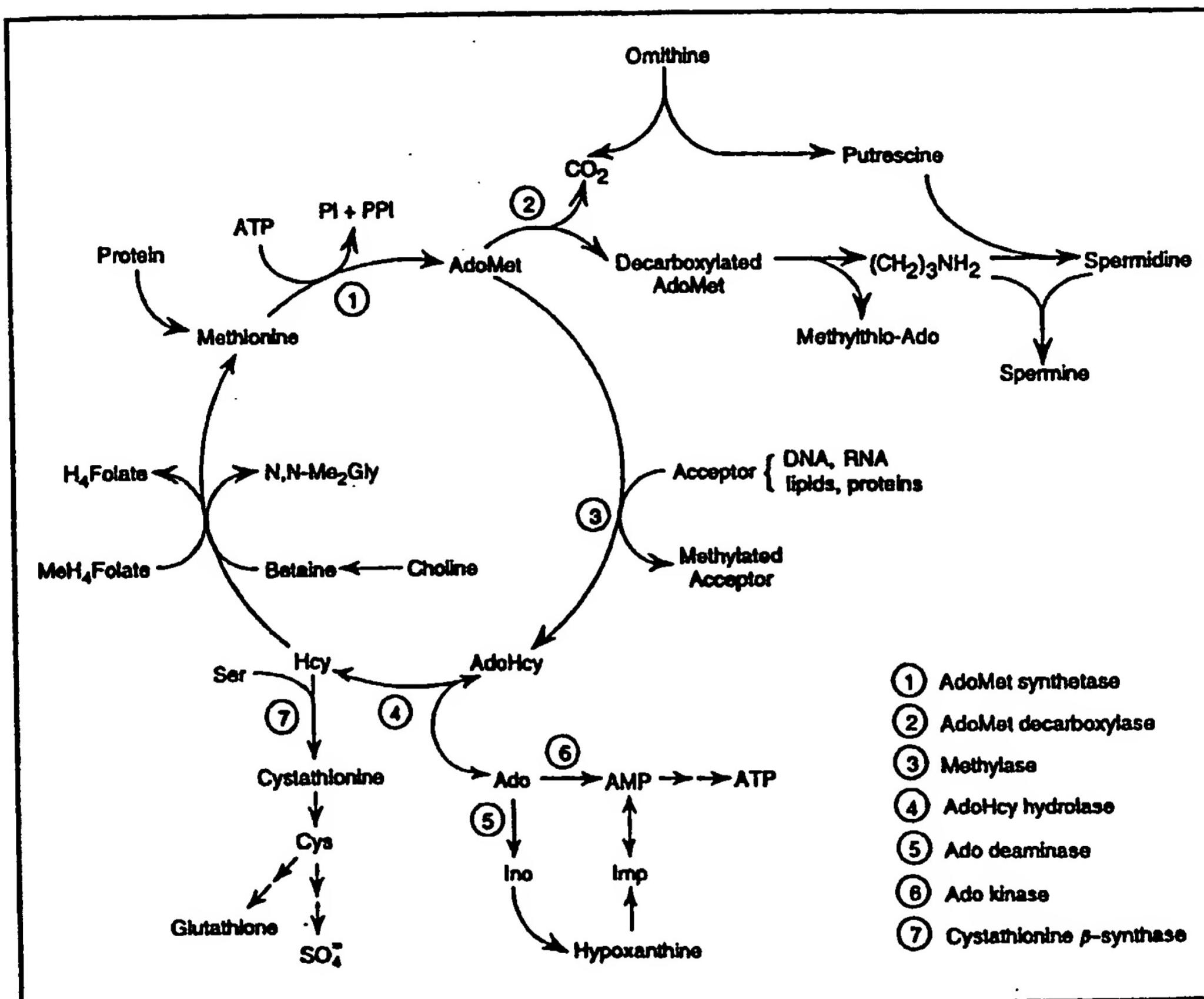


FIG 6

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RAP-PCR Method

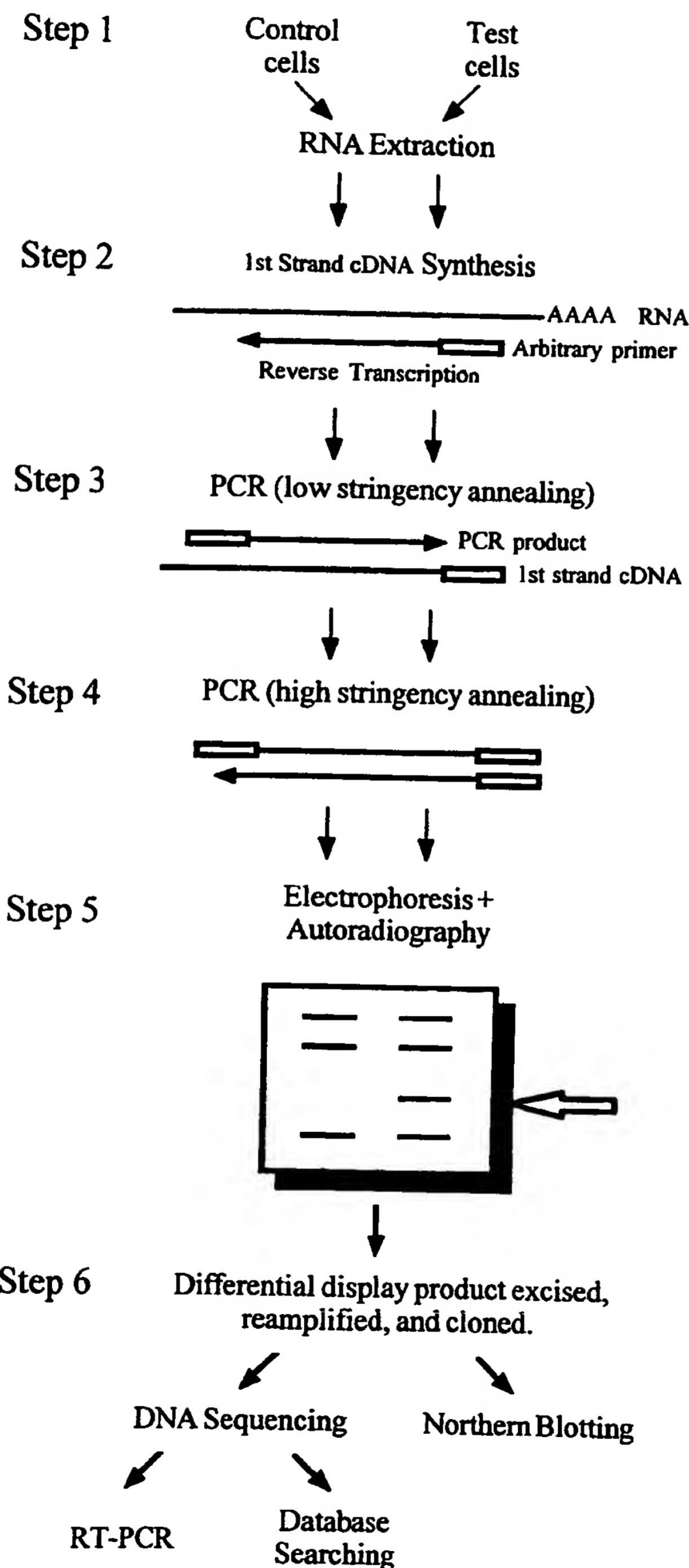


FIG 7

INTERNATIONAL SEARCH REPORT

International Application No.
PCT/NZ 97/00133

| A. CLASSIFICATION OF SUBJECT MATTER | | |
|---|---|--|
| Int Cl ⁶ : C12N 9/14, 15/55 | | |
| According to International Patent Classification (IPC) or to both national classification and IPC | | |
| B. FIELDS SEARCHED | | |
| Minimum documentation searched (classification system followed by classification symbols) See Electronic Database Box | | |
| Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched See Electronic Database Box | | |
| Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) Genebank, EMBL, Swissprot (Amino acids 177-314 of Fig. 1) EKQQTNSKGSSNFCVKNIKQAEFGRREIEEQDMSALISLRKRAQGEK PLAGAKIVGCTHTAQTAQTLALGAQCRWSACNIYSTQNEVAA ALAEAGVAVFAWKGESEDDFWWCIDRCVNMMDGWQANMILDDG | | |
| C. DOCUMENTS CONSIDERED TO BE RELEVANT | | |
| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
| X | Ogawa, H. (et al) "Amino acid sequence of s-adenosyl-L-homocysteine hydrolase from rat liver". <u>Proc. Natl. Acad. Sci. USA</u> , vol.84, No.719, 1987, pages 719-723 see entire document | 1-18 (in part) |
| X | Martin, C.H. (et al) "Complete sequence of the bithorax complex of Drosophila". <u>Proc. Natl. Acad. Sci. USA</u> , vol.92, pp 8392-8402, 1995 see particularly Table 1 | 1-18 (in part) |
| <input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C | | <input type="checkbox"/> See patent family annex |
| * Special categories of cited documents: "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier document but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed | | |
| Date of the actual completion of the international search 11 December 1997 | | Date of mailing of the international search report 16 DEC 1997 |
| Name and mailing address of the ISA/AU AUSTRALIAN INDUSTRIAL PROPERTY ORGANISATION PO BOX 200 WODEN ACT 2606 AUSTRALIA Facsimile No.: (02) 6285 3929 | | Authorized officer J.H. CHAN Telephone No.: (02) 6283 2340 |

INTERNATIONAL SEARCH REPORT

International Application N .

PCT/NZ 97/00133

| C (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT | | |
|--|--|-----------------------|
| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim N . |
| X | Kashir, J. (et al) "Amin acid sequence of S-Adenosyl-L-Homocysteine Hydrolase from <u>DICTYOSTELIUM DISCOIDEUM</u> as deduced from the cDNA sequence", <u>Biochem. and Biophys. Res. Com.</u> , vol.153, no.1, pages 359-364. see entire document | 1-18 (in part) |
| X | GOMI, T. (et al) "S-Adenosyl homocysteinase from Rat Liver", <u>J. Biol. Chem.</u> , vol.261, no.29, pages 13422-13425, 1986. see entire document | 1-18 (in part) |